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FEDERAL SPRING WHEAT GRADES—A DISCUSSION OF THEIR SHORTCOMINGS AND SUGGESTED REMEDIES

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If it be granted that the purpose of grades is to furnish standards of value to buyers and sellers, then the merits of grading systems should be measured by the success with which their grades provide indices of value. Inasmuch as spring wheat is nearly all used for milling into bread flour, our grading system should establish standards as indicative as possible of milling values or rather, in the final analysis, of baking values.

Altho our present system of federal grades has the merit of being explicit and of being impartially applied, it no longer serves the purpose of providing true indices of relative milling value, as a brief perusal of any record of wheat sales in the Minneapolis market will quickly reveal. Scarcely a day goes by that cars grading No. 3 or No. 4 Dark Northern do not bring more than the average being paid for No. 1 Dark Northern; and it is not only possible, but happens almost daily in every mixing house, that the blend of two or more cars of low grade wheat (say No. 3 Dark Northern) results in an equal number of cars of No. 1 Dark Northern, without anything having been done to the wheat other than mixing; a process that usually means an impairment, rather than an improvement in milling quality. It was originally intended that No. 3 Dark Northern, for example, should represent a standard inferior to No. 1 Dark Northern, but conditions are such that these standards no longer apply, or such a raise in grades would not have been possible without something having been done to improve the milling quality of the wheat. It is equally evident that the miller who can buy the two cars of No. 3 Dark for less money than some other miller would have to pay for the resulting two cars of No. 1 Dark, has a corresponding advantage. This has placed a serious handicap on those millers who are not in a position to afford

skilled buyers at terminal markets, laboratories, and other complicated equipment necessary to buy intelligently with the present system of federal grades. It is necessary for them to rely on the superior quality implied by our federal grades for a certificate labeled "No. 1" over a certificate labeled "No. 3." In our opinion it is the weakness of the present system of grades which has been materially responsible for the hard times recently experienced by those millers dependent for their supplies on terminal markets and elevator mixed wheat.

A certificate of grade on cars of wheat direct from country points has little significance, save perhaps as an excuse for a discount, for its grade is usually lowered on some one defect without its being raised for an excess of good points. This excess of good points, which does not in the least affect the grade, may and almost certainly will cause the car to bring a premium over an elevator mixed car (one usually a minimum, grade considered, in all points). This means that each country run car must, if the seller is to secure its real value, be sold on sample, in which case the only function of the grade is to emphasize its weakest point to the buyer, without stressing its strongest point to the seller, a state of affairs which necessarily means that the grade often provides a mere excuse for a discount.

With the advancement of research in cereal chemistry and the introduction of new cleaning machinery, the present system of grading fails to represent true values and country run cars must be sold by sample at our terminal exchanges. This requires that both buyers and sellers employ highly paid experts and establish laboratories in order that they may gauge the approximate milling value of wheat. This means that the public must not only stand the expense of our complicated inspection system, but must also stand the cost of the buying and selling experts and laboratories. If our system of grades furnished accurate descriptions of the wheat, or even better, gave grades which were proportional to the milling value, then we could dispense with all or part of this extraneous expense. We could also do away with the "scalping" of cash wheat, a practice made possible only by the extremely wide ranges of value for each grade, and one to which the Federal Trade Commission made violent objection as being suspiciously redolent of fraud.

The shortcomings of the present grades may be summarized as follows:

1. By permitting a single blemish to lower the grade without affording an opportunity to have this offset by marked excellence in some other property, we make possible wide ranges in milling value —and in price—within each grade.

2. The notation "dark" as an index of baking strength is practically valueless. It should be replaced with a notation giving protein content in percentage. Protein content is today a factor of quite as much importanch in baking value as test weight. Both buyers and sellers know from their protein maps the approximate protein content of country run wheat, but practically all elevator mixed wheat is today sold on protein certificate, and is usually determined in such manner that the buyer has little or no protection against fraud. Our grading system should take cognizance of a property universally acknowledged by the trade as of prime importance. At times premiums paid for each per cent of protein have run as high as 8% of the value of the wheat. This factor alone accounted at one time for a range of 20 cents per bushel within the same grades.

3. The present tolerances of imperfections are arranged in such an arbitrary fashion as to force uneconomic and expensive mixing at the terminals, a needless expense and detrimental to the millers dependent for their wheat on terminal elevators. A specific instance would be the profit to be derived from mixing very heavy, say 62 pound irrigated wheat grading No. 1 Northern, with bright colored but light weight wheat (say 54 pound) and grading No. 4 Dark Northern, with a resulting grade of No. 1 Dark Northern. This is uneconomic, for it is impossible to clean a blend of extremely heavy and extremely light weight wheat without heavy loss, and it is difficult both to temper and to mill it. It penalizes heavily the miller who, by buying this mixture, thinks he is securing the very best milling wheat obtainable.

4. The present distinctions between dockage and foreign material should be abolished, for to the small miller without cleaning machinery dockage becomes inseparable foreign material, and lowers milling value; while to the large mill with modern cleaning machinery inseparable foreign material, kingheads excepted, is no longer inseparable, but is dockage.

5. Adulteration by mixing in inferior varieties of wheat is today profitable on all sales of low grade wheat. The grading system should so be arranged as to make the maintenance of purity in the mixtures highly desirable.

Proposed Changes

The need of modifying the present system of grades should be perfectly evident from the foregoing. The system outlined below is put forward, not with any implication that it is the only possible system, but as a system which will meet for the most part the objections previously enumerated.

In making up a grading system, it is first necessary to list the properties affecting milling and baking value. In order of importance, these would seem to be about as follows:

1. Foreign material
2. Test weight
3. Protein content
4. Moisture content
5. Damage from heating
6. Damage other than heat
7. Admixtures of other varieties of wheats.

Under average conditions, a phrase admittedly difficult to define, these factors seem to affect values about as follows:

Foreign material.—As stated previously no distinction should be made between "inseparable" foreign material (with the exception of kingheads) and dockage. Both should lower the value of the gross mixture by 1% for each 1% of foreign material or dockage, the theory being that the cost of freight and cleaning is about offset by the value of the screenings. Foreign material that is truly inseparable, such as rye, has some value, so that the penalty of 1% should be ample to make it an object to market only clean wheat. It is felt, however, that the presence of more than 20% of foreign material should render the car of sample grade, as such a foul mixture is quite obviously unfit for milling unless cleaned. As kingheads seem to be truly inseparable it is suggested that in determining foreign material, each 1% of kingheads should count as 2% of foreign material, so that any sample containing more than 10% of kingheads would be of sample grade. In general, however, the penalty of 1% loss of price for each 1% of foreign material would provide sufficient incentive to clean all wheat that is very dirty.

Test weight.—An examination of the prices paid for light weight wheat on the rust crops of the Northwest shows that a penalty of about 1% of the price seems to be exacted for each ¼ pound deficiency in test weight.

Protein content.—The premiums paid for protein vary from year to year, but the average of recent years seems to be around 4% of the price for each 1% fluctuation in the protein content.

Moisture content.—Up to the point where an excess of moisture begins to impair the keeping properties, each extra percentage of moisture obviously lowers the value 1%. The keeping properties begin to decline when the moisture exceeds 14%. It is suggested, therefore, that the penalty for each percentage of moisture up to 14% be 1%; between 14 and 15%, 2%; and between 15 and 16%, 4%.

Any wheat containing more than 16% should be sold by sample, as its keeping properties are dangerously low.

Heat damage.—Discounts for heat damage are difficult to determine, so an arbitrary scale is suggested by which the value is lowered 1% for each ¼ of 1% of damage. Where there is more than 2½% of damage from heating, the wheat should be of sample grade. The limit under the present system is 3%.

Damage from causes other than heating.—A reasonable impairment in value for each 1% of damage would seem to be about 1%. In Canada the chief reason for discount from No. 1 Northern to No. 3 Northern is the presence of frosted wheat, 7% being allowed. Under normal conditions this seems to result in a price discount of about 7%. The delivery discount of 3% on Winnipeg futures is 8 cents per bushel. The presence of more than 10% of damaged wheat should reduce the wheat to sample grade.

Admixtures of other varieties of wheats.—Penalties for admixtures of other varieties of wheats must be more or less arbitrary, as the impairment of value depends in large measure upon the intended use. An impairment of 1% in value for each 2% addition of other varieties seems roughly to be the prevailing discount, except for the marked aversion to red durum, due to its milling properties being so entirely different from those of spring wheat. Red durum admixtures should be treated as tho the red durum were inseparable foreign material. However, when more than 20% of the mixture consists of varieties other than spring, it should be sample grade or a mixed notation should be added.

Our proposed grading system would then be arrived at by taking two extremes, one, a car of "perfect" wheat, and the other, a car of minimum milling value. To the car of maximum value we would arbitrarily assign a grade of 100, and to the car of minimum value a grade of 0. By this we do not necessarily mean that the car of minimum milling value is worthless, as it may have value for chicken feed, but we do mean that it is worthless for milling purposes. For intermediate grades we can interpolate for each property, using as a scale the percentages given above by which variations in the essential properties affect milling value. We can make a table, such as the one below, which will show the properties of the samples of maximum and minimum milling value, and the percentages of impairment of milling value for each property between these extremes.

Property	Maximum	Minimum	Impairment from Max. to Min.
Foreign material, per cent	0	20	20
Test weight, pounds	65	50	20
Protein content, per cent	15	10	20
Moisture content, per cent	10	16	10
Heat damage, per cent	0	2½	10
Other damage, per cent	0	10	10
Admixture of other wheats, per cent	0	20	10
Total impairment, per cent			100

In making up our actual grade, and to facilitate interpolation in the table given above, the inspection department would be furnished with a table showing the discounts from 100 which they were to make for each blemish. Such a table, but in somewhat more extended form, might be as follows:

TABLE OF DISCOUNTS

Discounts in per cent	Dockage and for. mat. in per cent under	Test weight per bushel in lbs.	Protein in per cent	Moisture in per cent under	Heat damaged in per cent under	Other damaged in per cent under	Other wheats in per cent under
0	0.	65+	15+	10.00	0	0	0
1	1.	64.25	14.75	10.50	.25	1.0	2.0
2	2.	63.50	14.50	11.00	.50	2.0	4.0
3	3.	62.75	14.25	11.50	.75	3.0	6.0
4	4.	62.00	14.00	12.00	1.00	4.0	8.0
5	5.	61.25	13.75	12.50	1.25	5.0	10.0
6	6.	60.50	13.50	13.00	1.50	6.0	12.0
7	7.	59.75	13.25	13.50	1.75	7.0	14.0
8	8.	59.00	13.00	14.00	2.00	8.0	16.0
9	9.	58.25	12.75	14.25	2.25	9.0	18.0
10	10.	57.50	12.50	14.50	2.50	10.0	20.0
11	11.	56.75	12.25	14.75	....	....	....
12	12.	56.00	12.00	15.00	....	....	....
13	13.	55.25	11.75	.....	....	....	....
14	14.	54.50	11.50	15.25	....	....	....
15	15.	53.75	11.25	.....	....	....	....
16	16.	53.00	11.00	15.50	....	....	....
17	17.	52.25	10.75	.....	....	....	....
18	18.	51.50	10.50	15.75	....	....	....
19	19.	50.75	10.25	.....	....	....	....
20	20.	50.00	10.00	16.00	....	....	....
Sample grade	20.+	50.00—	10.00—	16.00+	2.50+	10. +	20.0+

In using this table the inspector would note each property, add the discounts in question, and subtract the result from 100. This would give the grade.

EXPLANATORY EXAMPLES

(1) What is the numerical value of No. 3 dark northern, 56-pound test weight, 14.5% protein, 11.5% moisture?

Discount from 100%

Test weight, 56 pounds	12%
Protein, 14.5%	2%
Moisture, 11.5%	3%
Total discount	17%

Numerical value = 100% — 17% = 83%

(2) What is the numerical value of No. 2 dark northern, 57-pound test weight, 11.0 protein, 14.0% moisture, 1% kingheads, 3% damaged, 9% winter wheat.

Discount from 100%

Test weight, 57 pounds	10%
Protein, 11.0%	16%
Moisture, 14.0%	8%
Kingheads, 1%	2%
Damaged, 3%	3%
Winter wheat, 8%	4%
Total discount	43%

Numerical value = 100% — 43% = 57%

It will at once be objected that such a system would be too cumbersome, too expensive, and too impractical for country use. However, as each one of the factors considered is of prime importance in determining value, the trade virtually determines these properties accurately today, using, however, the high priced experts referred to above. It would be far cheaper, and far more to the advantage of both producer and ultimate consumer, to have these determinations made in an impartial laboratory under government supervision, than loosely by "experts" of varying degree. It is obvious that the country elevator operator can not analyze for protein each wagon load of grain brought in, any more than he can run a moisture test on the spot, but he will know after one or two carlot shipments have arrived at the terminal, about what these contents will be, just as today he does not know in buying, how much of a premium each wagonload will bring over the minimum of that grade at Minneapolis, until he has made one or two shipments, and found out in what part of the range of prices for each grade, his wheat belongs. Today no one tells him that his wheat runs 12.25 protein, but he quickly finds out that it brings a premium of 2 cents, 4 cents, or whatever it may be over minimum No. 1 Dark Northern. It is rare that the country elevator has time to count out the tenths of one per cent of foreign material present, or the percentages of varieties of wheat other than hard red spring. In actual practice he learns from his shipments

about what to grade his wheat. So it would be with the proposed system, except that at the terminal we would have a scientific system, under which any one would be perfectly safe in buying and selling by certificate.

It is quite conceivable that such a system as that proposed might not entirely eliminate ranges of value within each grade. Thus all wheat grading 70 might not bring the same price. It is quite possible that there might be a deficiency of high protein or high test weight wheat, and that the premiums paid for protein might go to 8% for each per cent of protein instead of being 4% as proposed, and the same might be true, but to a lesser degree, for test weight. In that case each miller in buying would simply specify the protein content and test weight and the premium he was willing to pay. There would be no need of his ever seeing the wheat if the government certificate certified that the protein and test weight were satisfactory. It is realized that the quality of the protein as well as the quantity is a price making factor, but in comparison with the quantity it is so negligible, that it is believed that millers would neglect to consider it in making their bids, except that they might specify certain points of origin noted for their low quality as being excluded. It is doubtful, however, if this would occur except possibly on crops in which the quality of the protein varied widely; and even in this case, it is not believed that ranges of price for wheats of the same grade would be particularly large, certainly not comparable with those prevailing today.

Even those readers who would regard the system outlined above as impracticable, will in all probability admit that it would furnish a very satisfactory index of milling value. Let us, then, analyze the present grading system in the light of the proposed one—trying to find out the maximum and minimum values possible for the present grades under the proposed system. This is well illustrated in the following table:

Dark Northern	Maximum grade	Minimum grade	Possible range in milling value
No. 1	100	63	37
No. 2	99	52	47
No. 3	98	44	54
No. 4	96	30	66
No. 5	95	Sample	95

The preceding table is arrived at in detail by using the discounts listed in the following table:

	No. 1 Dk. Nor. Discounts,		No. 2 Dk. Nor. Discounts,		No. 3 Dk. Nor. Discounts,		No. 4 Dk. Nor Discounts,		No. 5 Dk. Nor. Discounts,	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Foreign matter	0	0	2	0	3	0	5	0	7	0
Test weight	9	0	11	0	13	0	16	0	20	0
Protein	20	0	20	0	20	0	20	0	20	0
Moisture	4	0	5	0	6	0	10	0	10	0
Heat damage	0	0	1	1	2	2	4	4	SG	0
Damage	2	0	4	0	7	0	10	0	SG	0
Other wheat	2	0	5	0	5	0	5	0	5	5
Total discount	37	0	48	1	56	2	70	4	—	5

Let us repeat once more just what these figures mean. If 100 represents the range in milling value between the best cars available and the poorest that can be used for milling, then our present federal grades include the wide ranges indicated above. No. 1 Dark Northern may apply to cars anywhere in the top 37% of this range, while No. 5 Dark Northern may include everything from sample grade to within 5% of the top.

In actual practice the ranges in value of country run wheat are by no means as great as this would seem to indicate, as it is practically impossible for a country elevator to make the mixtures possible under the federal grades. Such mixtures can be made only at the terminals. This is the chief reason why the larger mills are willing to buy from terminal elevators only as a last resort, and why the small mills dependent for their wheat on terminal elevators receive mixtures from which it is almost impossible for them to produce a satisfactory flour.

No. 1 Dark Northern wheat at Minneapolis is now deliverable at 2 cents over the contract price. From the last table it is shown how the range in milling quality of No. 1 Dark Northern is from 63 to 100%. It is evident that the terminal operator is not going to deliver on contract any better wheat than he has to, in fact any No. 1 Dark put out by him will be as close to the 63% mark as he dares to make it. Country run No. 1 Dark Northern wheat would very rarely be under 70% and would usually be between 75 and 90%. From this it is evident why we have heavy cash premiums, and the sole reason why they are not greater is that the dry land farmer raising really choice wheat does not by any means get the premium for his wheat to which its heavy weight, high protein, and low moisture content entitles it. The fact of the matter is that the sole reason the farmer does not receive these higher premiums is that the grading system does not point out to the seller the virtues of his wheat, nor are these better qualities pointed out to the smaller mills, who are unable to afford

their own expert buyers. Moreover, in actual practice the few millers able to maintain their skilled buyers at terminal markets, are in position, by paying a small premium, to secure wheat of relatively superior quality.

If the Secretary of Agriculture decided to exercise his power and install the system outlined above, he would no doubt find contract markets willing to alter the grades deliverable on spring wheat contracts for future delivery, and make the contract grade wheat grading 100, with other grades to apply at discounts proportional to the grade. Thus wheat grading 90 would be applicable at 90% of the contract price, etc. This would greatly extend the range of deliverable wheat, but would in all probability work out so that it would be highly to the advantage of the elevators to deliver the very highest grading wheat they could find, as the discounts on grades would probably be somewhat severe, as the lower grades would probably be taken by feeders at prices very much better than millers would be willing to pay. It is quite certain that there would not be the reluctance on the part of millers that there now is to take delivery of wheat, as the incentive to mix up the minimum blends now necessary for delivery purposes would be gone. This might have an important result, as it would probably enormously broaden the volume of future trading, thereby giving both producer and consumer more stable markets.

It will be noted that practically the only mixing which would give a result other than the average of the grades to begin with, would be that of wet and dry wheat. It is felt that in this case this should be permissible, as there is an actual improvement in the warehousing properties of the wheat. It is conceivable that occasional cars grading sample grade might be blended at a profit, but in no case would any raising of the grade result from the blending of two cars of the same grade, whatever their individual properties, with the exception noted above.

It is believed that an analysis of federal grades on other varieties of wheats will reveal the same defects as brought out above and that the suggested system outlined, with slight modifications, would apply equally to these other wheats.

Even if the system outlined is not that finally adopted, let us by all means so alter our present grades that the new ones will be fairly indicative of milling value, and not a needless expense to both consumer and producer.

VISCOSITY AS A MEASURE OF GLUTEN QUALITY[1]

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It has long been recognized that the properties of wheat flour are extremely variable and that certain types of wheat grown under certain environmental conditions are superior for the manufacture of yeast leavened bread, while other types of wheat grown under similar or different environment are more adapted to the manufacture of pastry flours.

In spite of the immense amount of research expended upon the strong and weak flour problem, we are still far from a complete knowledge of all the factors involved, in fact the complexity of the problem is becoming more and more apparent.

Rumsey[2] has recently shown that the enzymatic activity of wheat flours may be the limiting factor in determining the loaf volume rather than any property of the gluten proteins. It is thus highly probable that the gluten proteins are in many instances blamed for poor quality when in reality the yeast is unable to produce the desired loaf volume because of inadequate diastase activity. This consideration makes it extremely difficult, if not impossible, to correlate differences in loaf volume with gluten quality unless the baking experiments are so conducted as to insure the presence of an adequate amount of diastase in all instances. So far as I am aware, no laboratory is following this procedure. We can therefore rule out the baking test as a reliable index of gluten quality.

The need for an accurate measure of gluten quality has stimulated the invention of a variety of mechanical devices which have been used more or less extensively, but no one of which has been universally adopted by either the scientific investigators or by the trade. Probably one reason why none of these instruments has been universally adopted is that *gluten quality* is not the only factor measured by the instrument, consequently the results can not be interpreted directly in terms of quality, nor can the values obtained with one flour be correlated accurately with the results obtained with another flour. We need, therefore, some accurate quantitative measure of gluten quality. Recent investigations indicate that viscosity measurements may afford

[1] Published with the approval of the Director as Paper No. 459, Journal Series, Minnesota Agricultural Experiment Station.

[2] Rumsey, L. A. The diastatic enzymes of wheat flour and their relation to flour strength. American Institute of Baking, Bul. 8. 1922.

such a measure, and it is the purpose of this paper to discuss briefly the principles underlying determinations of the viscosity of flour suspensions and their interpretation so far as gluten quality is concerned.

Wood[3] and Wood and Hardy[4] were the first to point out that the wheat flour proteins are typical emulsoid colloids.[5] Their investigations accordingly introduced into flour studies the methods used in colloid research. The result of such studies has been to indicate the importance of the colloidal behavior of the wheat proteins and strongly to suggest that the differences in gluten quality may be due to differences in the colloidal state.[6] Among the more characteristic of the properties of emulsoid colloids are the high viscosities attained by sols of relatively low concentrations and the great changes in viscosity which may be produced by what would appear to be relatively slight changes in environment.[7]

Lüers and Ostwald[8] were the first to examine systematically the viscosity of flour suspensions. Unfortunately these workers did not include both strong and weak flours in their experiments, but rather reasoned from analogy as to what factors are involved in flour strength. It has been pointed out elsewhere[9] that reasoning from analogy is a rather dangerous procedure when the material in question is an emulsoid sol or gel.

In addition, these authors used a viscosimeter of the Ostwald type which has been shown to be unsuited to an exact study of the viscosity of flour-water systems.[10]

In the studies on the viscosity of flour suspensions which have been in progress in this laboratory for the last four years, use has been made of the torsion type of viscosimeter.[11] It was found that such an

[3] Wood, T. B. Jour. Agr. Sci., Vol. 2, pp. 139-160, 267-277 (1907).

[4] Wood, T. B., and Hardy, W. B. Proc. Roy. Soc. London (B), Vol. 81, pp. 38-43 (1908).

[5] For those who are wholly unfamiliar with colloid theory and nomenclature, reference may be had to Wo. Ostwald and M. H. Fischer, "An introduction to theoretical and applied colloid chemistry," John Wiley & Sons, New York. 1917.

[6] It would take us too far afield to discuss the evidence upon which this statement is based. The reader is referred to J. Agr. Res., Vol. 13, pp. 389-418 (1918); J. Phys. Chem., Vol. 26, pp. 101-136 (1922); Vol. 27, pp. 481-492; 567-576; 674-684; 771-788; 982-987 (1923). Res. Bul. 19, Minn. Agr. Exp. Sta. (1924).

[7] See Wo. Ostwald, "The importance of viscosity for the study of the colloidal state," Proc. Faraday Soc., Vol. 2, pp. 34-46 (1913), reprinted (in German) in Kolloid Zeit, Vol. 12, pp. 213-222 (1913).

[8] Koll, Z., Vol. 25, pp. 82-90, 177-196, 230-240 (1919); Vol. 26, pp. 66-67 (1920); Vol. 27, pp. 34-37 (1920).

[9] Gortner and Doherty, J. Agr. Res., Vol. 13, pp. 389-418 (1918); and Sharp and Gortner, J. Phys. Chem., Vol. 26, pp. 101-136 (1922).

[10] Sharp and Gortner, Res. Bul. 19, Minn. Agr. Exp. Sta. (1924).

[11] A MacMichael viscosimeter has been used almost exclusively altho the Wallace and Tiernan viscosimeter has been recently found to be satisfactory. It is probable that any properly designed instrument based upon the torsion principle will give comparable results.

instrument affords a rapid measure of the viscosity of flour suspensions and that the results may be readily duplicated from time to time. The data of several studies have already been published.[12]

The significant finding in these experiments is that *viscosity may be used as a measure of gluten quality*. Unfortunately there appears to have been some misinterpretation of this statement and it is the purpose of this paper to point out the procedure which we have adopted for measuring viscosity and the method which is used to interpret the viscosity readings.

In the first place, the viscosity of a flour-in-water suspension is the resultant of several factors, among which *quantity of hydrophilic colloids* (proteins) and *quality of protein* are probably of major importance. Most of the remaining factors noted by Ostwald[7] can be more or less controlled. The inorganic electrolytes (ash) have a very profound influence on the viscosity of emulsoid systems, and as ash is extremely variable in flours, it is necessary to adopt special measures so that the ash content does not mask the desired information. The soluble ash is accordingly removed by a preliminary leaching of the flour sample with water, as noted below.

Both the *quantity* and *quality* factors vary from flour to flour, so that the absolute viscosity values are not necessarily related to the quality of the gluten. In other words, it is perfectly possible to have two flours differing in nitrogen content and in gluten quality in which the flour which consistently shows the higher viscosity value actually contains the poorer quality of gluten. For this reason, the absolute viscosity values must be corrected in such a way as to yield a figure which varies only with gluten quality.

This correction may be made by finding the viscosities of flour-water suspensions containing various concentrations of flour and then plotting the logarithms of the viscosities as abscissa and the logarithms of the flour concentration as ordinates. When this is done, it is found that the values lie on a straight line. The slope of this line in respect to the axis of abscissa or *the increment of increase of the logarithm of the viscosity per increment of increase of the logarithm of flour concentration determines the slope of the line.*

The equation for such a curve is

$$\text{log. viscosity} = a + b\ (\text{log. concentration})$$

where a and b are constants, a determines the position of the line on the viscosity axis at zero flour concentration and b is the tangent of

[12] The complete data are given by Sharp and Gortner. "Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength." Res. Bul. 19, Minn. Agr. Exp. Sta. (1924). The data, greatly condensed, are presented in J. Phys. Chem., Vol. 27, *loc. cit.* (1923).

the angle which the line makes with the horizontal. In other words, *b is directly related to the slope of the line and may be taken as a numerical value of gluten quality.* The value for b may be measured from the plotted line[13] or the constants may be calculated mathematically by the method of least squares[14] using the formulae

$$a = \frac{\Sigma(x).\ \Sigma(xy) - \Sigma(x^2).\ \Sigma(y)}{[\Sigma(x)]^2 - n\,\Sigma(x^2)}$$

$$b = \frac{\Sigma(x).\ \Sigma(y) - n\,\Sigma(xy)}{[\Sigma(x)]^2 - n\,\Sigma(x^2)}$$

where Σ = sum of values
x = log of flour concentration used
y = log of viscosity found
n = number of observations

TABLE I

Flour No.	Air dry flour grams	Dry flour* grams	Viscosity M°	Log. grams flour (x)	Log. viscosity (y)	x^2	xy
8045	12	10.546	23	1.0233	1.3617	1.047143	1.393427
	15	13.182	56	1.1199	1.7482	1.254176	1.957809
	20	17.576	133	1.2450	2.1239	1.550025	2.644255
	23	20.212	219	1.3056	2.3404	1,704591	3.055626
Σ				4.6938	7.5724	5.555935	9.051117
8132	10	8.7670	42	0.9428	1.6232	0.888872	1.530353
	12	10.5204	79	1.0220	1.8976	1.044484	1.939347
	15	13.1505	138	1.1189	2.1399	1.251937	2.394334
	18	15.7806	203	1.1981	2.3075	1.435444	2.764616
	21	18.4107	281	1.2650	2.4487	1.600225	3.097605
Σ				5.5468	10.4169	6.220962	11.726255

*It has been found that it is not necessary to calculate the logarithm on the dry basis. The same values for the constant b are obtained when the values for the air-dry flour are used.

Table I shows representative calculations for two flours differing markedly in gluten quality. When we substitute in the formula for b given above we have

$$\text{Flour 8045 } b = \frac{4.6938 \times 7.5724 - 4\ (9.051117)}{(4.6938)^2 - 4\ (5.555935)}$$

$$= \frac{-\ 0.661137}{-\ 0.191982} = 3.44$$

$$\text{Flour 8132 } b = \frac{5.5468 \times 10.4169 - 5\ (11.726255)}{(5.5468)^2 - 5\ (6.220962)}$$

$$= \frac{-\ 0.850664}{-\ 0.337820} = 2.52$$

[13] In case this is done, care must be taken to plot equal increments of logarithms on the same scale on both axes.

[14] Mellor, J. W. Higher mathematics for students of chemistry and physics. Longmans, Green and Co., London, 1916. Cf., pp. 326-330.

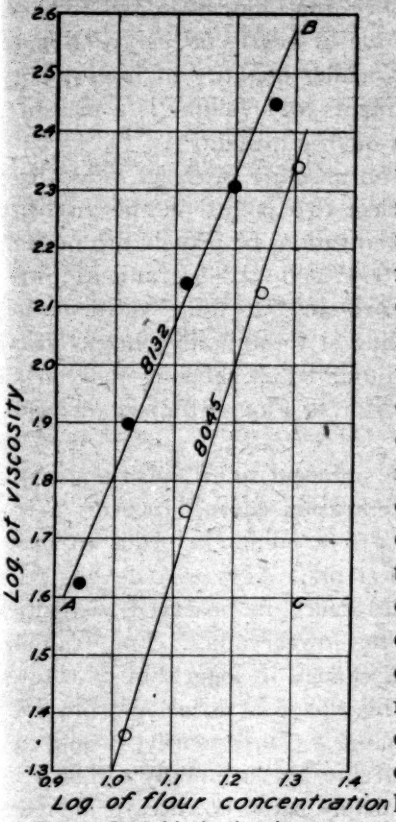

Fig. 1. Logarithmic viscosity- concentration curves of two flours differing in gluten quality.

In Figure 1 are plotted the experimental data for the two flours. The lines indicating the slope of the viscosity-concentration curve are drawn to lie at the angle calculated by the method of least squares. The extent to which the observed points deviate from this line is indicative of the experimental error which may occur. It will be noted by an inspection of the figure that it would be extremely difficult to determine the *exact slope* of the line if the line were drawn by relying solely on the judgment of the draftsman as to its relation to the observed points. Consequently any tangent measured from the charted data may be appreciably in error. The calculation of the tangent from the charted data can best be shown by reference to Figure 1. The tangent of the angle C A B (Flour 8132) is obtained by dividing the length of the line CB by the length of the line AC.

The constant for Flour 8045 indicates that the viscosity-concentration curve makes an angle of 73° 47′ with the axis of abscissa, while Flour 8132 gives an angle of 68° 21′, showing that *the quality of the gluten in Flour 8045 is superior to that in 8132 altho the absolute viscosity values of Flour 8132 are markedly higher at an equal concentration.* Flour 8045 contained 9.75 per cent of protein on the dry basis and Flour 8132 contained 14.27 per cent, so that the *quantity*[15] factor obscures the *quality* factor when absolute viscosities are compared but does not influence the quality constant as calculated by the above method. The loaf volumes of these two flours are of interest as a further indication that the gluten in Flour 8045 is superior in quality to the gluten of Flour 8132. *The two flours produced loaves*

[15] It is probable [Sharp and Gortner. J. Phys. Chem., Vol. 27, pp. 674-684 (1923)] that the glutenin content and not total protein content is actually the *quantity* factor. There is, however, some evidence that the ratio of glutenin to total protein does not vary as widely as has been supposed so that, for rough calculations, the total protein content may be used.

having almost identical volumes (1840 cc. for Flour 8045 and 1880 cc. for Flour 8132) *altho Flour 8132 contains nearly 50 per cent more protein than Flour 8045.* The much smaller quantity of high quality protein was approximately equal, so far as loaf volume is a measure, to a much larger quantity of medium quality protein.

The effect of the *quantity* factor on absolute viscosity values can be shown in another way. If we select two points on the viscosity concentration curves where equal concentrations of protein are present (as, for example, 15 grams of Flour 8045 and 10.248 grams of Flour 8132, both containing 1.4625 grams protein) we find by calculation from Figure 1 that the viscosity reading at these flour concentrations is 82° for Flour 8045 and 69° for Flour 8132, showing that on an equal protein basis Flour 8045 is superior to Flour 8132, as was indicated by the quality constant *b*.

Ordinarily two or three points are sufficient to establish a straight line. In the case of a viscosity-concentration curve, however, it has been found necessary to use four or five points in order to mask erroneous results due to experimental errors. As we are dealing with a logarithmetic function, a slight difference in observed viscosities may produce an appreciable error in the lower values. For example, the range 12° M. to 16° M. makes a change in logarithm of 0.1249, whereas at the higher viscosities we may have a variation from 200° M. to 267° M. to produce a similar change. Consequently, if only two or three points are taken the slope of the line is unduly affected by the lower viscosity reading. It is probably better to discard all readings lower than 25° M. in order to avoid excessive errors.

The method for preparing flour samples and determining viscosities has been standardized in this laboratory as follows:

Apparatus needed:

- Torsion viscosimeter
- Triple beam balance
- 1000 cc. graduated cylinder
- 100 cc. graduated cylinder fitted with rubber stopper
- 1000 cc. Pyrex liter Erlenmeyer flasks fitted with rubber stoppers
- 2 stirring rods
- 1 burette—2 cc. graduated in ½ cc.
- 20% lactic acid
- Distilled water

Care must be taken to insure *clean* apparatus and absence of contamination during all processes since *traces* of electrolytes will alter the viscosity values.

Weigh out on the triple beam balance 12, 15, 18, and 21 gram portions[16] of flour into 4 dry 1000 cc. Erlenmeyer flasks.

Add 100 cc. of distilled water to each flask. Insert stopper *immediately after adding water* and shake vigorously until a smooth suspension of flour is formed. Then add 900 cc. more of distilled water and shake occasionally (8 to 10 times) during 45 minutes. Let stand quiet for 15 minutes. Carefully decant the supernatant liquid from the flour residue in the bottom of the flask and discard the liquid. Add 500 cc. of distilled water to the residue, shake, let stand quietly for 15 minutes and decant carefully as before, discarding the liquid. Pour the residue into the 100 cc. graduated cylinder and rinse flask, pouring rinsings into cylinder, finally bringing volume of liquid in cylinder to 100 cc. Stopper cylinder and mix contents by inverting several times. Pour contents into cup of viscosimeter, suck liquid down to constant level point[17] (constant immersion of bob),[18] add 0.5 cc. of 20% lactic acid,[19] stir contents to mix uniformly, insert plunger of viscosimeter, take viscosity reading. Check this reading a second time.

Wash out cup, dry, and repeat with the next sample.

Plot the logarithms of the concentrations and viscosities as ordinates and abscissas on graph paper. A straight line should result. The quality of the gluten is the tangent of the angle which this line makes with the axis of abscissa. This value can be approximately measured from the graph or can be calculated by the formula given above.

SUMMARY

1. It has been pointed out that absolute viscosities of flour-water suspensions may yield results which may be wrongly interpreted because the viscosity is the resultant of two factors, the *quantity* and *quality* of the glutenin present in the flours.

2. A method has been described for determining a constant which is apparently characteristic of the *quality* of the glutenin present in wheat flour.

[16] If the flour contains less than 9 per cent protein, it is advisable to use 15, 18, 21, 24; or 18, 21, 24, 27 gram portions.

[17] A convenient device for producing a constant level can be made by fastening a glass tube held at a right angle through a hole in a straight edge. The straight edge is then laid across the top of the viscosimeter cup. When suction from a water pump is applied to the glass tube, the liquid in the cup is lowered to the level to which the tube is immersed in the liquid. This level can be fixed by sliding the glass tube up or down through the straight edge.

[18] For the MacMichael viscosimeter we have found it convenient to use the 2 cm. cylindrical bob, with a 2 cm. immersion and a rotation of the cup of 76 r. p. m., using a No. 30 wire.

[19] This will bring the flour-water suspension to approximately a pH of 3.0, at which maximum viscosity is attained.

GLYCEROL AS AN AID IN ASHING FLOUR

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The A. O. A. C. official method of flour ashing[1] is accurate but has the disadvantage of the long time necessary to obtain a fairly white ash. The following method was found to give similar results in much less time:

Mix 5 grams of flour in the ashing dish with 10 cc. of a glycerol-alcohol solution made from equal volumes of each. Clean the mixing rod with a small piece of ashless filter paper. Ignite the paper with the sample. After the alcohol has burned off, place in an electric furnace held at approximately 575°C. (dull red) and burn to a white ash. Place in an efficient desiccator. Weigh when cool.

Use redistilled glycerol yielding no residue after burning, otherwise make a correction for the blank obtained from 10 cc. of the glycerol-alcohol solution.

The glycerol is the effective agent in shortening the ashing period. When the glycerol is burned off there is produced a bulky and porous char with considerably more surface than that from flour burned alone. The alcohol is added to the glycerol in order to obtain a solution that is more mobile and more accurately and conveniently measured than glycerol alone. The alcohol-glycerol solution also mixes more readily with the flour.

Typical results from a straight flour obtained by the proposed method and the official A. O. A. C. method are as follows:

PROPOSED METHOD				A. O. A. C. METHOD			
Time	Color of Ash	%	%	Time	Color of Ash	%	%
1½ hrs.	Almost white	0.472	0.476	4½ hrs.	Quite dark	0.472	0.468
2 hrs.	White	0.464	0.468	5 hrs.	Dark gray	0.472	0.468

[1] Book of Methods, 1920, A. O. A. C., p. 71.

WHEAT AND FLOUR STUDIES. I. PROTEOLYTIC ENZYMES OF FLOUR I. AUTO-DIGESTION OF FLOUR MILLED FROM FROZEN AND NON-FROZEN WHEAT HARVESTED AT VARIOUS STAGES OF MATURITY

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Introduction

The belief by flour investigators that the proteolytic enzymes present in the flour have no effect on the proteins of the flour, at least during the period of yeast fermentation, has been frequently expressed. This belief is based partly on the fact that flour of high grade digested with water for periods ranging up to about twenty-four hours shows practically no change in the proteins during this period. This would indicate that proteolytic enzymes, if present in high-grade flour, are present only in small amounts. On the other hand it is quite possible that the enzymes may cause changes in the colloidal character of the proteins which would definitely affect flour strength, without causing any appreciable change as shown by ordinary protein solubility analysis.

In attempts to determine the effect of the proteolytic enzymes of the flour on the amounts of the proteins themselves, no one, apparently, has allowed the enzyme to act long enough to show any pronounced proteolytic digestion of high-grade flours.

In the investigation reported here, four main points are under consideration. (1) Will the proteolytic enzymes of wheat flour digest the flour proteins if given sufficient time? (2) What proteins of the flour are attacked by the enzymes present in the flour? (3) Does the proteolytic activity vary with the maturity of the wheat from which the flour is milled? (4) Does the subjection of the immature wheat to temperatures slightly below freezing, corresponding to a light frost, affect the activity of the proteolytic enzymes? A review of the literature indicates that these points have not previously been investigated.

Balland (4) demonstrated the presence of a protein-splitting enzyme in sprouted-wheat flour and in bran. He used a solution

[1] Published with the approval of the Director.

[2] A part of this work was presented to the chemistry department of the Montana State College as an undergraduate thesis by Miss Roma Elmer.

obtained by extracting 100 grams of sprouted-wheat flour or bran with 250 cc. of water to make up the doughs in the crude gluten determination. The longer a dough so prepared was allowed to stand before washing out the gluten, the less was the amount of gluten obtained.

Vines (16) found that if either fibrin or Witte-peptone was added to a water extract of wheat germ a distinct tryptophane reaction was obtained after a few hours.

Baker and Hulton (2) state:

"That the appearance of white of egg when treated in the presence of toluene with an aqueous extract of flour was considerably altered, the albumin being disintegrated and the solution becoming milky. . . . The analytical results showed that there was no increase of soluble nitrogen in aqueous flour extract after digestion for four hours at 30°C. in the presence of white of egg. This was confirmed by the absence of the tryptophane reaction when flour water and egg albumin were kept at 37°C. for sixteen hours. When flour extract was allowed to act on its separated gluten for 18 hours at 37°C. there was practically no evidence of solution and no alteration in the appearance of the gluten."

These authors auto-digested nine flours with chloroform-saturated water for twenty hours at 37°C. Four of the filtrates gave no tryptophane test, four a slight test, and one a strong test. The experiment was repeated with the addition of Witte-peptone and all flours gave the reaction. They conclude that this indicates the presence of erepsin.

Baker and Hulton finally state:

"Since the 'strength' of flour is probably largely dependent on the character of the gluten, and erepsin being without action on insoluble proteins, we must look further for the cause of any possible gluten hydrolysis, which if it occurs during the doughing and baking process would almost certainly affect the size or shape of the loaf."

Ford and Guthrie (6) demonstrated the presence of a proteolytic enzyme in flour by showing that either the flour or an extract would liquify gelatin. These authors attempted to show an increase in amino nitrogen by applying Sorensen's formol-titration but without success.

"The use of polypeptides after Abderhalden was also inadequate, and failure attended other processes devised by ourselves, as for example the determination of the amount of change in the nature of the nitrogenous matter in the extracts of wheat, etc."

Swanson and Tague (13) studied the proteolytic enzymes in flour by determining the amino nitrogen, by means of Sorenson's formol-titration, after periods of auto-digestion ranging up to 8 weeks. They

found that the presence of salts and other proteins affected the amount of amino nitrogen formed.

Stockham (12) used the gelatin-liquifying method. He found in general that the lower the grade of the flour the more rapid the liquification, also that the proteolytic activity of straight flours decreased with age so that after 8 months the liquifying power in a number of cases was not demonstrable. Low-grade flours and flours from sprouted wheat were, however, active after 15 months and the activity in sprouted wheat was still demonstrable after 3 years. He concluded that aging decreases proteolytic activity. Stockham cut wheat kernels transversely into two portions. The flour and feeds milled from the germ-end portion of the kernel liquified gelatin relatively rapidly, while the flour and feeds from the blossom end liquified gelatin slowly and the high-grade flour showed practically no liquifying power. From the study of the gelatin liquifying power of a large number of flours he concludes, "It appears that the differences between wheats of different classes or varieties could not be attributed, to a very great extent if at all, to proteolytic activity of the wheat."

Collatz (5) using the viscosity method described by Gortner and Sharp (7) (8) was able to demonstrate that the addition of various small amounts of malt flour to ordinary baking flours produced a pronounced decrease in the imbibitional capacity of the flour proteins. The conditions of the experiment were such as to indicate that the malt flour would have some effect on the flour proteins during ordinary dough fermentation.

Of the workers mentioned, Balland (4), Swanson and Tague (13), and Collatz (5) seemed to be the only ones who used the proteins of the flour itself as a substrate. It is conceivable that the enzymes of the flour would not digest foreign proteins at the same rate as the natural flour proteins; and as it is their effect on the proteins of the flour in which we are mainly interested, any method of studying the enzymes of the flour using the proteins of the flour as the substrate would be preferable to methods in which the ability of the enzyme to digest a protein foreign to the flour is studied. It is also possible that the enzymes of the flour digest the various proteins of the flour at different rates. Sharp and Gortner (10) found that the glutenin of wheat is the protein responsible for the increased hydration capacity produced by acids. The work of Collatz on the proteolytic enzymes of malt flour indicates that the enzymes acted on the glutenin. This work does not give light as to their effect on the other proteins of the flour. However, if the proteolytic enzymes of malt flour markedly

decrease the hydration capacity of the glutenin, the effect on the resulting bread would be very detrimental whether the enzymes had any effect on the other proteins or not.

Experimental

In order to ascertain whether or not the digestion of the proteins of the flour by the enzymes present could be demonstrated if the enzymes were allowed to act over a long enough period of time, a preliminary experiment was performed. A baker's patent flour, No. 201, milled from Montana wheat was chosen. The flour had aged about two or three months. The method used in studying the proteolytic activity of the flour was to autolyze with toluene-saturated water and determine the protein fractions on the basis of their solubility after various time intervals. The autolysis was carried out at a temperature of 35°C.

For each auto-digestion mixture, 50 grams of flour were transferred to a weighed 500 cc. volumetric flask, and the flask was then filled to the mark with distilled water and again weighed. In the case of flour No. 201 this required approximately 464.0 grams of water. The mixture was then transferred to a 500 cc. Erlenmeyer flask, 1 cc. of toluene added, and the flask placed in an air thermostat at 35°C. The flasks were, of course, kept stoppered and were shaken to insure thoro mixing and to prevent lump formation.

The aliquots for analysis were removed with a 25 cc. pipette after a thoro shaking of the mixture. The pipette used had a tip with a very large opening, which was necessary in order to remove the aliquot before any appreciable settling had taken place. As seen from the method of preparation of the digestion mixture, a 25-cc. aliquot would contain 2.5 grams of the original flour and approximately 23.2 cc. of water. The methods utilized in determining the protein solubility fractions were as follows:

1. **Potassium sulphate-soluble proteins.**—The autolyzing suspension was thoroly shaken and a 25-cc. aliquot immediately transferred to a centrifuge bottle. To the material in the centrifuge bottle, 25 cc. of 10 per cent potassium sulphate solution and 1.8 cc. of distilled water were added, thus the 2.5 grams of flour removed were treated with 50 cc. of 5 per cent potassium sulphate. The extraction was carried out by shaking in a mechanical shaker for one hour after which the material was centrifuged and the nitrogen in a 25-cc. aliquot of the clear supernatant liquid determined by the Kjeldahl method. The nitrogen was converted to protein by the use of the factor 5.7 and the result expressed in percentage of flour on the dry basis. The

remainder of the supernatant liquid was decanted into a 25-cc. graduated cylinder and the volume recorded. The protein determined in this manner is called throughout the paper the 5 per cent potassium sulphate-soluble fraction. It is seen that this solution would contain any albumin and protein split products soluble in water, in addition to the true globulins.

2. **Alcohol soluble-protein in the residue after extraction with potassium sulphate solution.**—To the residue in the centrifuge bottle enough 70 per cent alcohol was added to make 75 cc. of liquid. This was accomplished by adding 50 cc. of alcohol by means of a pipette and the remainder by filling the 25-cc. graduated cylinder with an amount of 70 per cent alcohol equivalent to the amount of potassium sulphate extract remaining after removing the 25-cc. aliquot for the nitrogen determination as recorded above. The amount of liquid remaining in the residue after potassium sulphate extraction was approximately 3.0 cc. This would dilute the alcohol somewhat and would still contain a small amount of protein, but probably not enough to influence the results, especially after the greater portion of the potassium sulphate-soluble protein had been removed. The material was extracted by shaking for one hour in a mechanical shaker and it was then centrifuged. Nitrogen was determined on a 50-cc. aliquot of the supernatant liquid, converted to protein by the use of the factor 5.7, and expressed on the basis of percentage of the original moisture-free flour.

3. **Glutenin.**—According to the solubilities of the various proteins of flour, the residue after the two above extractions should be glutenin. The glutenin was not determined, however, by making a protein determination on the residue but by subtracting from the total protein in the flour (total nitrogen $\times$ 5.7) the sum of the potassium sulphate-soluble protein and the alcohol-soluble protein as obtained above. This method of determining glutenin was used by Sharp and Gortner (11), and is not the method given by the Association of Official Agricultural Chemists (1). It is deduced from the findings of Bailey and Blish (3).

4. **Amino nitrogen.**—A 25-cc. aliquot of the autolyzing flour and water mixture was removed and placed in a small flask. To this was added 1.25 cc. of a 20 per cent solution of sodium tungstate and 0.35 cc. of distilled water. The mixture was shaken, allowed to stand a few minutes, and 4 drops of concentrated sulphuric acid were added. This mixture was shaken, allowed to stand a short time, centrifuged, and the amino nitrogen in duplicate 2-cc. aliquots was determined with the micro Van Slyke (15) amino nitrogen apparatus. For com-

parison with the values obtained for the proteins, the amino nitrogen was also multiplied by the factor 5.7.

The principal errors are probably the following: Settling of the material during the taking of the aliquot for analysis. Coalescing of the particles of the gluten, due to shaking, these being taken up in unequal amounts in the aliquots or adhering to the sides of the digestion flask. Other work did not permit the carrying out of the determinations in duplicate except in the few instances mentioned. It is believed, however, that enough points along the digestion curve were determined practically to eliminate the effect of the few points which may be in considerable error. The amount of flour used for the experiment and in the aliquots was smaller than would have been desirable for more accurate results, but the quantity of flour available limited the amount which could be used for this work.

The methods described really divide the proteins contained in the flour into three fractions. The gliadin has been considered by several workers to be the most important protein of the flour. Lately Sharp and Gortner (10) (11), and Woodman (17) have brought forth new evidence calling attention to the importance of glutenin. The remaining proteins are ordinarily present in the flour in relatively small amounts, nevertheless they are also considered of importance by some investigators who hold the belief that the baking quality of a flour is indicated by the percentage of protein in the water-soluble form. As it is hard to make any accurate quantitative separation of the globulin, albumin, and protein split products of flour this was not thought worth while, consequently they were all determined together as one fraction and are called throughout this paper the 5 per cent potassium sulphate-soluble fraction. The use of 5 per cent potassium sulphate solution for the first extraction has an additional advantage in that gliadin is less soluble in it than in distilled water.

The preliminary experiment, using flour No. 201, was carried out in triplicate flasks. The results are given in Table I and are expressed graphically in Figure 1. It should be remembered that any error in determining either the alcohol or the potassium sulphate-soluble proteins will also affect the value obtained for glutenin. Thus in Table I, Fig. 1, week 0, flask A, the alcohol-soluble protein is undoubtedly too high, causing a corresponding lowering in the value obtained for the glutenin. This method of difference for determining the glutenin was adopted because time was not available for the actual carrying out of a determination on the residue. It was ascertained, however, that results obtained for glutenin by difference were in substantial agreement with those obtained by a nitrogen determination of the residue.

TABLE I

PROTEIN FRACTIONS DETERMINED ON FLOUR NO. 201 AFTER VARIOUS PERIODS OF AUTO-DIGESTION. TEMPERATURE OF DIGESTION 35°C.

This determination was carried out in triplicate flasks, designated A, B, and C in the table.

Time of auto-digestion Weeks		K_2SO_4-Sol. protein on the basis of total Flour	Crude protein	Alcohol-Sol. protein on the basis of total Flour	Crude protein	Glutenin on the basis of total Flour	Crude protein	Amino nitrogen expressed as protein (N×5.7) on the basis of total Flour	Crude protein
		%	%	%	%	%	%	%	%
	A	2.10	15.5	8.17	60.4	3.26	24.1	0.05	0.4
0	B	1.91	14.1	6.26	46.3	5.36	39.6	0.05	0.4
	C	1.90	14.1	6.45	47.9	5.18	38.0	0.03	0.2
	A	2.42	17.9	3.70	27.3	7.41	54.8	0.25	1.8
1	B	2.74	20.3	3.73	27.6	7.06	52.1	0.24	1.8
	C	2.91	21.5	5.53	26.1	7.09	52.4	0.17	1.2
	A	3.10	22.9	3.62	26.8	6.81	50.3	0.35	2.6
2	B	4.11	30.4	3.79	28.0	5.63	41.6	0.34	2.5
	C	4.42	32.7	4.08	30.1	5.03	37.2	0.32	2.4
	A	4.57	33.7	3.54	26.2	5.42	40.1	0.40	2.9
4	B	4.96	36.7	2.32	17.1	6.25	46.2	0.42	3.1
	C	5.20	38.4	3.93	29.1	4.40	32.5	0.44	3.2
	A	5.04	37.3	3.53	26.0	4.96	36.7	0.49	3.6
5	B	4.74	35.0	3.51	26.0	5.28	39.0	0.45	3.3
	C	4.88	36.1	3.89	28.8	4.76	35.1	0.45	3.3

An examination of Table I and Figure 1 would indicate that while the experimental error is apparently large yet the results show a definite trend. The potassium sulphate-soluble protein increases steadily up to the fourth week with no appreciable increase the fifth week. The amino nitrogen shows a very great increase the first week with a diminishing rate of increase to the fifth week. The gliadin fraction shows a pronounced decrease the first week with possibly a slight decrease to the fifth week. This marked decrease of the gliadin fraction at the end of the first week is not all accounted for by the increase in the potassium sulphate-soluble fraction and consequently shows up as an increase in the glutenin fraction. After the end of the first week the glutenin fraction decreases steadily, the decrease being accounted for almost quantitatively by the increase in the potassium sulphate-soluble fractions, until at the end of the 5 weeks period the amount of protein in the glutenin fraction is practically the same as that at the start.

While it is apparent that the experimental error is rather large in these determinations, in spite of this drawback definite changes in the proteins are indicated by differences in their solubilities. There is a marked increase in the potassium sulphate-soluble fraction which would contain most of the protein split products. A decided decrease in the alcohol-soluble fraction indicates that the gliadin is readily attacked while the effect of the enzyme on the glutenin is left in

doubt, but the results indicate that it escapes the attack of the enzyme as far as a solubility analysis shows.

As these results seemed to offer sufficient encouragement, the investigation was extended to flours milled from slightly frosted and non-frosted wheats harvested at various stages of maturity.

A portion of a field of Marquis spring wheat was purchased from a farmer living within a few miles of the Montana Agricultural Experiment Station. This wheat was grown under a system of dry farming. At two- or three-day intervals during the development of the kernel, portions of the wheat were harvested. In order to stop growth as soon as possible only the heads were harvested. The gathering of bulk samples of the heads was facilitated by the use of an improvised comb made by driving headless nails into a piece of wood. Enough heads were secured at a time to fill eight 24-pound flour sacks. Four of these sacks of heads were subjected to freezing temperatures before drying.

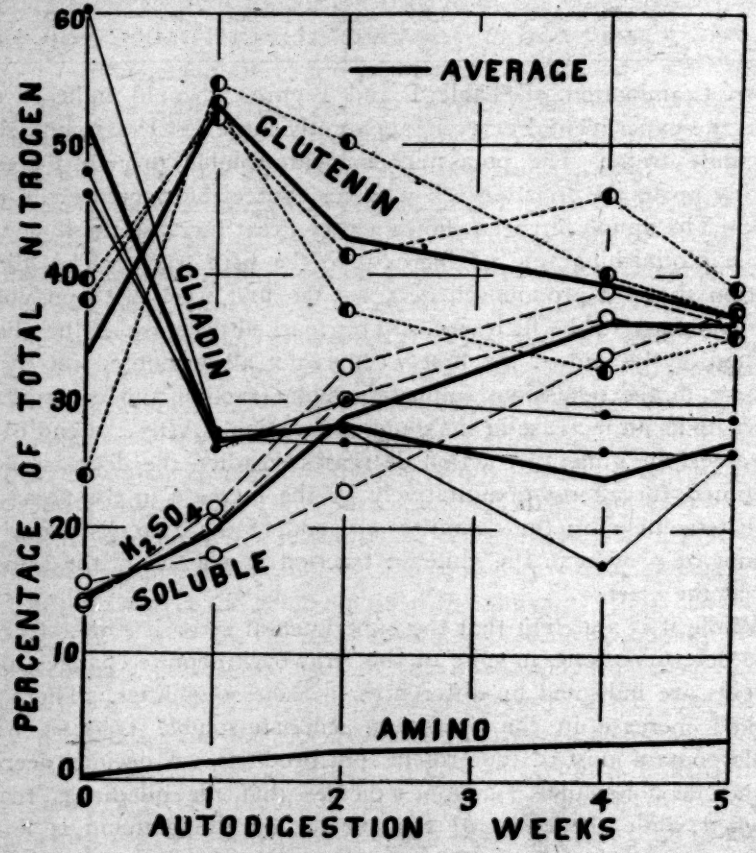

Fig. 1. Variation in results obtained by carrying out the determinations in triplicate flasks.

TABLE II

PROTEIN (N×5.7) EXTRACTED FROM FLOUR BY 5 PER CENT POTASSIUM SULPHATE SOLUTION DETERMINED AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on basis of original moisture-free flour. Even laboratory numbers indicate the frozen aliquots.

Lab. No.	Approximate age of kernel	Ash	Flour at beginning	Total protein	Time interval, weeks 0	1	2	3	4	6	16
	Days	%	pH	%	%	%	%	%	%	%	%
S-105	13	1.40	6.13	11.77	3.85	4.18	4.58	4.95	5.24	4.41	5.98
S-106	13	1.43	6.22	11.90	3.75	3.96	4.40	4.43	4.91	5.30	5.42
S-107	15	1.02	6.23	11.47	3.18	3.41	3.74	3.84	3.57	4.11	4.07
S-108	15	1.18	6.24	11.41	3.10	3.12	3.47	3.41	3.66	3.95	3.86
S-109	17	1.02	6.25	11.05	2.84	3.07	3.54	3.86	4.19	4.79	5.40
S-110	17	1.10	6.26	11.11	3.10	3.84	5.69	7.01	7.50	7.95	8.47
S-111	20	0.88	6.30	10.67	2.46	2.88	4.02	5.59	5.88	3.54	7.66
S-112	20	1.02	6.13	10.70	2.56	2.81	3.28	3.82	3.51	3.79	4.09
S-113	22	0.91	6.16	10.62	2.69	2.87	3.32	4.19	3.78	4.25	5.00
S-114	22	0.83	6.25	10.23	2.32	2.41	2.65	3.29	2.91	3.01	3.48
S-115	24	0.78	6.28	10.62	2.22	2.17	2.36	2.94	2.58	2.50	3.25
S-116	24	0.80	6.23	11.60	2.19	2.19	2.52	3.09	2.60	1.83	2.94
S-117	27	0.67	6.17	11.65	2.24	2.21	2.54	2.00	2.59	2.95	2.89
S-118	27	0.78	6.18	11.40	2.21	2.27	2.62	2.57	2.74	1.05	2.28
S-119	29	0.75	6.18	11.76	2.18	2.36	2.43	2.30	2.48	2.90	2.89
S-120	29	0.72	6.15	11.19	2.26	2.29	2.52	2.39	2.63	2.67	2.64
S-121	31	0.63	6.21	12.09	2.18	2.68	2.64	1.79	2.96	1.82	3.78
S-122	31	0.62	6.29	12.00	2.06	2.21	2.40	2.17	2.92	2.43	2.70
S-123	34	0.52	6.21	12.13	1.96	1.91	2.34	1.95	2.21	3.37	2.27
S-124	34	0.52	6.22	11.64	1.95	2.03	2.66	2.11	2.29	....	2.54

The freezing was accomplished by placing the sacks in one of the cans used for freezing water in a commercial artificial ice plant. The temperature of the brine surrounding the cans was maintained at about —10° to —11°C. The sacks were kept under the freezing conditions for a minimum of 20 hours, by which time a thermometer placed in the center of the sack gave a reading of —2° to —3°C.

The heads from the eight sacks were dried in the sun by spreading them out in trays placed on a flat roof. After drying they were threshed and milled. It was rather difficult to obtain a good separation of flour in the more immature samples, as may be seen from the ash analysis given in Table II. The protein content of the wheat is below the average for this region. The kernels showed blisters only in the later stages, the number of blistered kernels in any of the samples not exceeding 35 to 40 per cent. In the most immature stages there was no difference in number of green kernels between the frozen and non-frozen samples. It is probable that freezing wheat in this way corresponds to a light frost and not a severe one; therefore conclusions drawn from this set of experiments might not hold in case of a severe frost. The conditions of freezing to which these samples were subjected, however, occur more frequently than do the more severe freezing temperatures.

The autolytic method previously described was used. The moisture content of the series of flours varied from 9.94 to 11.28 per cent with the exception of flour S-124 which had a moisture content of 12.05 per cent. The weight of water required to fill the 500 cc. volumetric flask after introducing 50 grams of flour was determined, using 4 flours falling in different places in the age series, and found to be 464.0, 464.2, 464.2, and 465.2 grams. In making up the remainder of the digestion mixtures 464.0 grams of water was added directly to 50 grams of flour contained in a 500 cc. Erlenmeyer flask. The results obtained are expressed in Tables II to V on the percentage basis of the original moisture-free flour. Table II also gives the age of the kernel in days, the ash content of the flour, the pH of a 1:5 flour-water suspension and the total protein content. The even numbered samples indicate the frozen aliquots, the odd numbered the non-frozen ones. Tables VI to IX express the results in terms of the percentage of total protein or total nitrogen in the various fractions.

Teller (14) analyzed wheat harvested at various stages of growth for gliadin, glutenin, edestin and leucosine, and amides. His results indicate an increase of gliadin and a decrease of glutenin as the kernel develops.

TABLE III

PROTEIN (N×5.7) SOLUBLE IN 70 PER CENT ALCOHOL AFTER PREVIOUS EXTRACTION WITH 5 PER CENT POTASSIUM SULPHATE SOLUTION. DETERMINED AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on the percentage basis of original moisture of free flour

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	2.60	2.44	3.06	1.91	1.44	0.56	0.78
S-106	2.73	2.61	2.53	0.86	0.82	1.04	0.72
S-107	3.27	2.96	2.37	2.25	1.94	1.42	1.33
S-108	3.13	3.11	2.55	2.45	1.87	1.41	1.32
S-109	3.22	3.13	2.68	2.68	2.13	1.59	0.82
S-110	3.10	3.26	1.93	1.44	1.27	0.79	0.76
S-111	3.44	3.33	0.55	1.30	2.31	1.42	0.93
S-112	3.20	3.11	2.54	2.22	1.92	1.59	1.01
S-113	3.16	3.13	2.28	0.98	1.99	1.41	0.69
S-114	3.55	3.14	2.55	3.06	2.23	2.00	0.85
S-115	3.47	3.22	....	3.02	2.24	1.87	1.04
S-116	3.47	2.94	2.66	2.90	1.89	1.59	1.00
S-117	4.25	3.12	3.10	2.99	2.59	2.14	0.95
S-118	4.10	3.24	2.79	2.57	2.42	1.91	0.87
S-119	4.14	4.07	3.45	2.48	3.29	1.79	2.44
S-120	4.26	....	....	2.77	2.79	2.44	1.91
S-121	4.36	2.93	3.84	3.74	3.23	2.37	1.38
S-122	4.30	3.33	3.73	3.97	3.12	2.37	1.60
S-123	4.19	3.57	2.95	3.22	3.21	2.47	1.41
S-124	4.23	3.51	4.03	3.26	3.64	2.70	1.90

He, however, determined the gliadin on a separate sample which would tend to give too high results for the gliadin and too low results for the glutenin, if compared with the method used by Sharp and Gortner (11). He apparently did not carry out the same series of analyses on the flour.

The data presented in Tables II to IX show the changes in the protein fractions of the flour milled from wheat harvested at various stages of growth. The values given for the beginning of the digestion period were all carried out in duplicate and when these failed to check they were repeated. The percentage of total nitrogen in the various fractions at the start, plotted against the age of the kernel in days, is given in Figure 2.

Figure 2 indicates that there is practically no change in the percentage of total nitrogen of the flour falling in the glutenin fraction, there is apparently a rather definite increase in the gliadin fraction, and a definite decrease in the potassium sulphate-soluble and amino nitrogen. In general the differences in analyses shown by the flour from frozen and non-frozen kernels are well within experimental error and justify the conclusion that freezing as carried out under these conditions had no effect on the distribution of the nitrogen as shown by solubility analyses of the proteins.

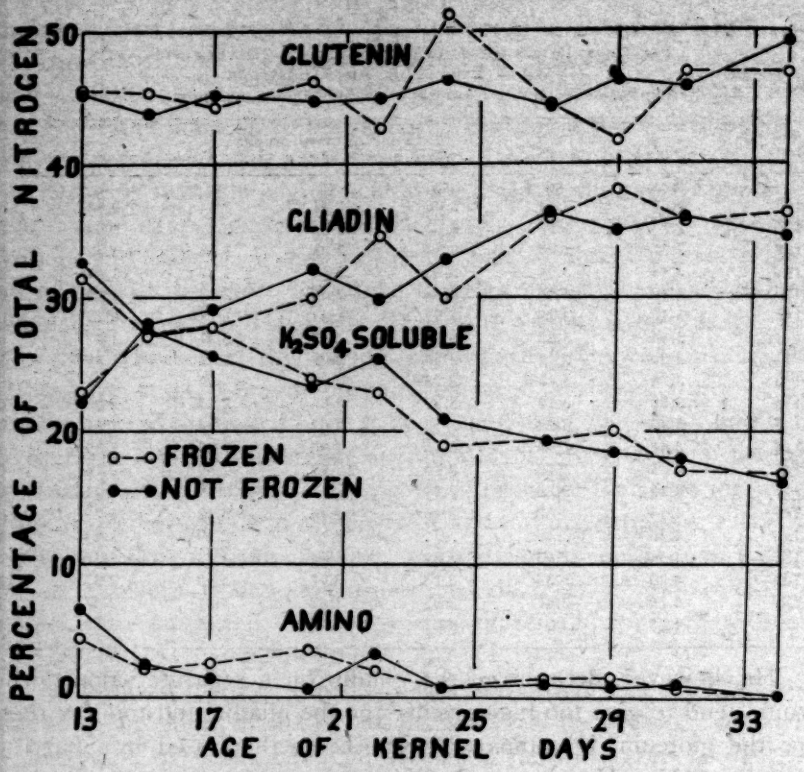

Fig. 2. Protein fractions of flour milled from frozen and non-frozen wheat harvested at various stages of growth.

The effect of age of kernel and of frost on the changes produced by proteolytic enzymes is shown graphically in Figure 3 which expresses the results given in Tables VI to IX. The age, in days, of the kernel is indicated by the numeral in the lower left-hand corner of each unit figure. With the exception of the flour from the kernels frozen at 17 days of age, which were abnormal in other respects, and of flour from the non-frozen kernels at 20 days of age, the glutenin fraction either remains constant or increases. This indicates no digestion of the glutenin by the enzymes present in the flour during the period under observation. The gliadin fraction decreased in every case as digestion progressed, while the potassium sulphate-soluble and amino nitrogen fractions increased in every case. The results indicate that the increase in the potassium sulphate-soluble and amino nitrogen fractions is not so marked in the flour milled from the more nearly mature kernels. The decrease in the gliadin fraction is roughly about the same throughout the period of kernel development studied.

TABLE IV

PROTEIN (GLUTENIN) IN FLOUR RESIDUE AFTER EXTRACTION WITH 5 PER CENT POTASSIUM SULPHATE SOLUTION FOLLOWED BY 70 PER CENT ALCOHOL, DETERMINED AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Values calculated by difference and expressed on the percentage basis of the original moisture-free flour

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	5.32	5.15	4.13	4.91	5.09	6.80	5.01
S-106	5.42	5.33	4.97	6.61	6.17	5.56	5.76
S-107	5.02	5.10	5.36	5.38	5.96	5.94	6.07
S-108	5.18	5.18	5.39	5.55	5.88	6.05	6.23
S-109	4.99	4.85	4.83	4.51	4.73	4.67	4.83
S-110	4.91	4.01	3.49	2.66	2.34	2.37	1.88
S-111	4.77	4.46	6.10	3.78	2.48	5.71	2.08
S-112	4.94	4.78	4.88	4.66	5.27	5.32	5.60
S-113	4.77	4.62	5.02	5.45	4.87	4.96	4.93
S-114	4.36	4.68	5.03	3.88	5.09	5.22	5.90
S-115	4.93	5.23	....	4.66	5.80	6.25	6.33
S-116	5.94	6.47	6.42	5.61	7.11	8.18	7.66
S-117	5.16	6.32	6.01	6.66	6.47	6.56	7.81
S-118	5.09	5.89	5.99	6.26	6.24	8.44	8.25
S-119	5.44	5.33	5.88	6.98	5.99	7.07	6.34
S-120	4.67	....	....	6.03	5.77	6.08	6.64
S-121	5.55	6.48	5.61	6.56	5.90	7.90	6.93
S-122	5.64	6.46	5.87	5.86	5.96	7.20	7.70
S-123	5.98	6.65	6.84	6.96	6.71	6.29	8.45
S-124	5.46	6.10	4.95	6.27	5.71	....	8.10

TABLE V

AMINO NITROGEN EXPRESSED AS PROTEIN (N×5.7) DETERMINED IN FLOUR AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on the percentage basis of the original moisture-free flour

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	0.78	1.09	1.10	1.15	1.35	1.51	1.42
S-106	0.51	0.82	0.86	0.96	1.14	1.17	1.13
S-107	0.29	0.51	0.53	0.61	0.71	0.70	0.58
S-108	0.24	0.46	0.47	0.51	0.63	0.61	0.58
S-109	0.17	0.45	0.48	0.62	0.74	0.83	0.88
S-110	0.29	0.59	0.92	1.34	1.69	1.82	1.83
S-111	0.06	0.29	0.40	0.72	1.07	1.13	1.22
S-112	0.38	0.58	0.61	0.72	0.73	0.71	0.70
S-113	0.34	0.51	0.59	0.70	0.81	0.88	0.89
S-114	0.20	0.32	0.37	0.40	0.51	0.51	0.60
S-115	0.09	0.15	0.20	0.25	0.28	0.29	0.35
S-116	0.09	0.17	0.20	0.27	0.29	0.29	0.31
S-117	0.14	0.24	0.24	0.24	0.30	0.33	0.35
S-118	0.21	0.29	0.30	0.36	0.41	0.41	0.46
S-119	0.11	0.19	0.22	0.32	0.44	0.35	0.53
S-120	0.18	0.23	0.32	0.32	0.39	0.37	0.35
S-121	0.14	0.22	0.26	0.35	0.42	0.40	0.45
S-122	0.10	0.16	0.18	0.39	0.28	0.26	0.23
S-123	0.03	0.06	0.10	0.19	0.16	0.15	0.15
S-124	0.01	0.08	0.14	0.21	0.20	0.18	0.70

TABLE VI

PROTEIN EXTRACTED FROM FLOUR BY 5 PER CENT POTASSIUM SULPHATE SOLUTION DETERMINED AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on basis of percentage of total protein

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	32.7	35.5	38.9	42.0	44.5	37.5	50.8
S-106	31.5	33.3	37.0	37.2	41.3	44.5	45.6
S-107	27.7	29.7	32.6	33.5	31.1	35.8	35.5
S-108	27.2	27.4	30.4	29.9	32.1	34.6	33.8
S-109	25.7	27.8	32.0	34.9	37.9	43.3	48.9
S-110	27.9	34.6	51.2	63.1	67.5	71.6	76.2
S-111	23.1	27.0	37.7	52.4	55.0	33.2	71.8
S-112	23.9	26.3	30.6	35.7	32.8	35.4	38.2
S-113	25.3	27.0	31.2	39.5	35.5	40.0	47.1
S-114	22.7	23.6	25.8	32.2	28.5	29.5	34.0
S-115	20.9	20.7	22.2	27.7	24.4	23.5	30.6
S-116	18.9	18.9	21.7	26.7	22.4	15.8	25.3
S-117	19.3	18.9	21.5	17.2	22.2	25.3	24.9
S-118	19.4	19.9	23.0	22.5	24.0	9.2	20.0
S-119	18.5	20.1	20.7	19.5	21.1	24.7	24.6
S-120	20.2	20.5	22.5	21.4	23.5	23.9	23.6
S-121	18.0	22.1	21.8	14.9	24.5	15.1	31.3
S-122	17.2	18.4	20.0	18.1	24.3	20.2	22.5
S-123	16.1	15.7	19.3	16.0	18.2	27.8	18.7
S-124	16.8	17.4	22.9	18.1	19.6	....	21.8

TABLE VII

PROTEIN SOLUBLE IN 70 PER CENT ALCOHOL AFTER PREVIOUS EXTRACTION WITH 5 PER CENT POTASSIUM SULPHATE SOLUTION DETERMINED AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on the basis of percentage of total protein

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	22.1	20.7	26.0	16.3	12.2	4.8	6.6
S-106	22.8	21.9	21.2	7.2	6.8	8.7	6.0
S-107	28.5	25.8	20.7	19.6	16.9	12.4	11.5
S-108	27.4	27.2	22.4	21.5	16.4	12.4	11.6
S-109	29.1	28.3	24.3	24.3	19.2	14.4	7.4
S-110	27.9	29.3	17.4	12.9	11.4	7.1	6.9
S-111	32.2	31.2	5.1	12.2	21.7	13.3	8.7
S-112	29.9	29.0	23.8	20.7	17.9	14.9	9.4
S-113	29.8	29.5	21.4	9.2	18.7	13.3	6.5
S-114	34.7	23.6	24.9	29.9	21.8	19.5	8.3
S-115	32.8	30.2	....	28.4	21.1	17.6	9.8
S-116	29.9	29.9	22.9	25.0	16.3	13.7	8.7
S-117	36.4	26.8	25.8	25.6	22.2	18.4	8.1
S-118	36.0	28.4	24.4	22.6	21.2	16.8	7.6
S-119	35.2	34.6	29.3	21.1	28.0	15.2	21.5
S-120	38.1	....	....	24.7	24.9	21.8	17.1
S-121	36.1	24.3	31.8	30.9	26.7	19.6	11.4
S-122	35.8	27.8	31.1	33.1	26.0	19.8	13.3
S-123	34.6	29.4	24.2	26.6	26.4	20.3	11.5
S-124	36.3	30.2	34.6	28.0	31.3	23.2	8.6

TABLE VIII

PROTEIN (GLUTENIN) IN FLOUR RESIDUE AFTER EXTRACTION WITH 5 PER CENT POTASSIUM SULPHATE SOLUTION FOLLOWED BY 70 PER CENT ALCOHOL DETERMINED AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on the basis of percentage of total protein

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	45.2	43.8	35.1	41.7	43.3	57.7	42.6
S-106	45.7	44.8	41.8	55.6	51.9	46.8	48.4
S-107	43.8	44.5	46.7	46.9	52.0	51.8	53.0
S-108	45.4	45.4	47.2	48.6	51.5	53.0	54.6
S-109	45.2	43.9	43.7	40.8	42.9	42.3	43.7
S-110	44.2	36.1	31.4	24.0	21.1	21.3	16.9
S-111	44.7	41.8	57.2	35.4	23.3	53.5	19.5
S-112	46.2	44.7	45.6	43.6	49.3	49.7	52.4
S-113	44.9	43.5	47.4	51.3	45.8	46.7	46.4
S-114	42.6	45.7	49.3	37.9	49.7	51.0	57.7
S-115	46.3	49.1	....	43.9	54.4	58.9	59.6
S-116	51.2	51.2	55.4	48.3	61.3	70.5	66.0
S-117	44.3	54.3	50.7	57.2	55.6	56.3	67.0
S-118	44.6	51.7	52.6	54.9	54.8	74.0	72.4
S-119	46.3	45.3	50.0	59.4	50.9	60.1	53.9
S-120	41.7	....	....	53.9	51.6	54.3	59.3
S-121	45.9	53.6	46.4	54.2	48.8	65.3	57.3
S-122	47.0	53.8	48.9	48.8	49.7	60.0	64.2
S-123	49.3	54.9	56.4	57.4	55.4	51.9	69.8
S-124	46.9	52.4	42.5	53.9	49.1	....	69.6

TABLE IX

AMINO NITROGEN EXPRESSED AS PROTEIN (N×5.7) DETERMINED IN FLOUR AFTER VARIOUS PERIODS OF AUTO-DIGESTION

Results expressed on the basis of percentage of total protein

Lab. No.	Time interval, weeks 0	1	2	3	4	6	10
	%	%	%	%	%	%	%
S-105	6.6	9.3	9.4	9.9	11.5	12.9	12.0
S-106	4.3	6.9	7.2	8.1	9.7	9.8	9.5
S-107	2.5	4.5	4.6	5.3	6.2	6.1	5.0
S-108	2.1	4.0	4.2	4.4	5.5	5.3	5.1
S-109	1.5	4.0	4.3	5.6	6.7	7.5	8.0
S-110	2.6	5.3	8.3	12.1	15.2	16.4	16.5
S-111	0.5	2.8	3.8	6.8	10.0	10.6	11.4
S-112	3.5	5.4	5.7	6.7	6.8	6.6	6.5
S-113	3.2	4.8	5.5	6.6	7.6	8.3	8.4
S-114	2.0	3.1	3.5	3.9	5.0	4.9	5.8
S-115	0.8	1.5	1.9	2.4	2.7	2.8	3.3
S-116	0.8	1.5	1.7	2.3	2.5	2.5	2.7
S-117	1.2	2.0	2.1	2.1	2.6	2.8	3.0
S-118	1.8	2.2	2.6	3.2	3.6	3.6	4.1
S-119	0.9	1.6	1.9	2.7	3.7	3.0	4.5
S-120	1.6	2.1	2.9	2.8	3.5	3.3	3.1
S-121	1.1	1.9	2.2	2.9	3.4	3.3	3.8
S-122	0.8	1.3	1.5	3.3	2.4	2.2	1.9
S-123	0.3	0.5	0.8	1.6	1.3	1.3	1.3
S-124	0.1	0.7	1.2	1.8	1.7	1.6	6.1

The work of various investigators indicates that the proteolytic enzymes are more concentrated in the branny and germ portions of the kernel, especially in the scutellum (9). If more of these portions are present in the flour in one case than in another, differences in proteolytic activity of such flours would be expected. As the proportion of bran coating to endosperm is greater in immature wheat, it would be expected that the ash content of the flour milled from immature wheat would be greater than in flour from mature wheat, owing to the inclusion of more of the bran in the flour fraction. On the other hand the ash of immature wheat is more uniformly distributed throughout the berry than that of mature wheat, hence the high ash of immature wheat flour does not necessarily mean low milling grade.

The yields of flour from the immature samples were, of course, less than from the mature ones. While the flour milled from the more immature stages apparently shows a greater proteolytic activity in some respects, the data do not justify the statement that this greater activity is due to a greater amount of enzymes in the endosperm, an inclusion of more of the branny layer, or to more easily severed chemical groups in the protein molecule.

In the flour milled from frozen and non-frozen wheat harvested on the 15th, 17th, 20th, and 27th days there is, perhaps, a wide enough range in the ash content to indicate a great enough difference in milling grade to affect the proteolytic activity of the flours. The agreement between the frozen and non-frozen samples of the 15th and 27th days could hardly be better, the 17th-day sample of frozen wheat was extremely abnormal in several respects and could not justly be used as a basis for comparison. The non-frozen sample, representing the 20th-day harvest, is also abnormal in its proteolytic activity. It does happen to contain less ash than the frozen sample, its behavior thus being the reverse from what might be expected.

The composition of the mixture after 10 weeks auto-digestion of the flours milled from frozen and non-frozen kernels of various ages, is shown in Figure 4. This figure should be compared with Figure 2 which gives the data for the beginning of auto-digestion. Figure 4 indicates that at the end of the digestion period the two principal protein fractions present, as shown by solubility analysis of the most immature wheat flour, are the glutenin and potassium sulphate-soluble fractions. The amount of gliadin is relatively small. As the kernel develops, the amount of protein in the potassium sulphate-soluble fraction decreases markedly and the protein in the glutenin fraction increases to a great extent. The amount of gliadin also increases. In the immature stages the gliadin is apparently broken down into

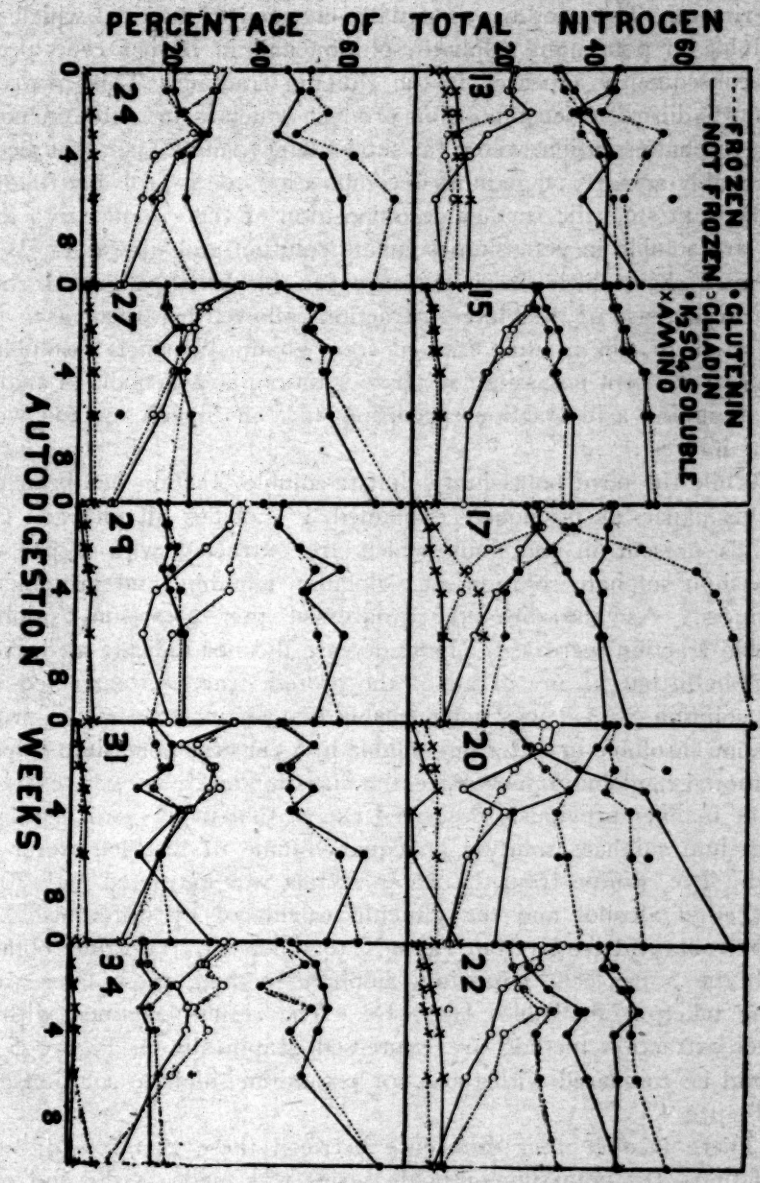

Fig. 3. Effect of auto-digestion on the protein fractions of flour milled from frozen and non-frozen wheat harvested at various stages of maturity. The numeral in the lower left-hand corner of each unit figure represents the approximate age of the kernel, in days.

substances which are soluble in potassium sulphate, while in the later stages decomposition did not progress this far in the time of the experiment. The gliadin was patially changed to material which was insoluble in potassium sulphate solution and in 70 per cent alcohol and consequently appeared in the glutenin fraction. This formation from gliadin of a decomposition product which is insoluble in potassium sulphate solution, water (as shown later), and 70 per cent alcohol is probably an early stage in its decomposition and is probably followed by the next step, the further decomposition of this product into those that are soluble in potassium sulphate solution and in water. While Figure 3 shows little or no evidence for this belief, Figure 1 shows first an increase of the glutenin fraction followed by a decrease. The subjection of this product formed from gliadin, which is insoluble in water, 5 per cent potassium sulphate solution, and alcohol, to analysis for its amino acid fractions according to Van Slyke's method would be of interest.

While the term potassium sulphate-soluble fraction has been used in this paper, as previously explained, it includes all nitrogen compounds present in the flour which are extracted with 5 per cent potassium sulphate solution, i.e., globulin, albumin, and protein split products. As auto-digestion progressed this potassium sulphate-soluble fraction increased. This increase did not indicate an increase in globulin but in any or all of the protein fractions extracted with this solution. As it was not probable that an increase in the protein fraction insoluble in water but soluble in 5 per cent potassium sulphate solution, i.e., globulin, took place, the digestion mixtures were extracted in the manner previously described except that in place of 5 per cent potassium sulphate solution an equal volume of distilled water was used. The residue from the water extract was extracted with 70 per cent ethyl alcohol and the glutenin calculated by difference. The results obtained are given in Table X, together with the values obtained with the 5 per cent potassium sulphate method, these later values being taken from Tables II to IX. The results obtained with the water-extraction method are expressed graphically in Figure 5 and should be compared with those for potassium sulphate solution given in Figure 4.

There is one other difference between these two sets of determinations, the potassium-sulphate series was made at the end of 10 weeks auto-digestion while the water-extract series was made at the end of 11 weeks auto-digestion, time and equipment not being available to make them both the same week. As most of the potassium-

TABLE X

(1) Protein (N×5.7) Dissolved in 5 Per Cent Potassium Sulphate Solution and (3) Protein Extracted from the Residue with 70 Per Cent Alcohol, Auto-Digestion Period 10 Weeks, Compared with (2) Protein Dissolved by Water and (4) Protein Extracted from the Residue with 70 Per Cent Alcohol, Auto-Digestion Period, 11 Weeks. Protein in the Final Residue (Glutenin) Calculated from the Total Protein by Difference Using in One Case (5) the Results Found in Columns (1) and (3) and in the Other Case (6) those in (2) and (4)

Results expressed in terms of percentage of flour, dry basis, and of total protein.

Lab. No.	K_2SO_4-Sol. (1)		H_2O (2)		GLIADIN After K_2SO_4 (3)		GLIADIN After H_2O (4)		GLUTENIN After K_2SO_4 (5)		GLUTENIN After H_2O (6)	
	Flour	Total protein	Flour	Total protein	Flour	Total protein	Flour	Total protein	Flour	Total protein	Flour	Total protein
	%	%	%	%	%	%	%	%	%	%	%	%
S-105	5.98	50.8	5.72	48.7	0.78	6.6	0.64	5.4	5.01	42.6	5.41	45.9
S-106	5.42	45.6	5.39	45.3	0.72	6.0	0.16	1.3	5.76	48.4	6.35	53.4
S-107	4.07	35.5	4.01	35.0	1.33	11.5	0.86	7.5	6.07	53.0	6.60	57.5
S-108	3.86	33.8	3.88	34.0	1.32	11.6	0.88	7.7	6.23	54.6	6.65	58.3
S-109	5.40	48.9	5.11	46.2	0.82	7.4	0.66	6.0	4.83	43.7	5.28	47.8
S-110	8.47	76.2	5.20	46.8	0.76	6.9	0.60	5.4	1.88	16.9	5.31	47.8
S-111	7.66	71.8	7.93	74.3	0.93	8.7	0.48	4.5	2.08	19.5	2.26	21.2
S-112	4.09	38.2	4.21	39.3	1.01	9.4	0.34	3.2	5.60	52.4	6.15	57.5
S-113	5.00	47.1	4.87	45.9	0.69	6.5	0.59	5.5	4.93	46.4	5.16	48.6
S-114	3.48	34.0	3.60	35.2	0.85	8.3	1.03	10.1	5.90	57.7	5.60	54.7
S-115	3.25	30.6	3.18	29.9	1.04	9.8	0.72	6.8	6.33	59.6	6.72	63.3
S-116	2.94	25.3	2.90	25.0	1.00	8.7	0.86	7.4	7.66	66.0	7.84	67.6
S-117	2.89	24.9	3.40	29.2	0.95	8.1	0.75	6.4	7.81	67.0	7.50	64.4
S-118	2.28	20.0	3.31	29.0	0.87	7.6	0.81	7.0	8.25	72.4	7.28	64.0
S-119	2.88	24.6	3.29	28.0	2.53	21.5	2.20	18.7	6.34	53.9	6.27	53.3
S-120	2.64	23.6	2.81	25.1	1.91	17.1	1.49	13.3	6.64	59.3	6.89	61.6
S-121	3.78	31.3	4.16	34.4	1.34	11.1	1.13	9.4	6.97	57.6	6.80	56.2
S-122	2.70	22.5	3.34	27.8	1.60	13.3	1.38	11.5	7.70	64.2	7.28	60.7
S-123	2.27	18.7	2.74	22.6	1.41	11.5	1.23	10.1	8.45	69.8	8 16	67.3
S-124	2.54	21.8	5.94	51.0	1.00	8.6	1.43	12.3	8.10	69.6	4.27	36.7

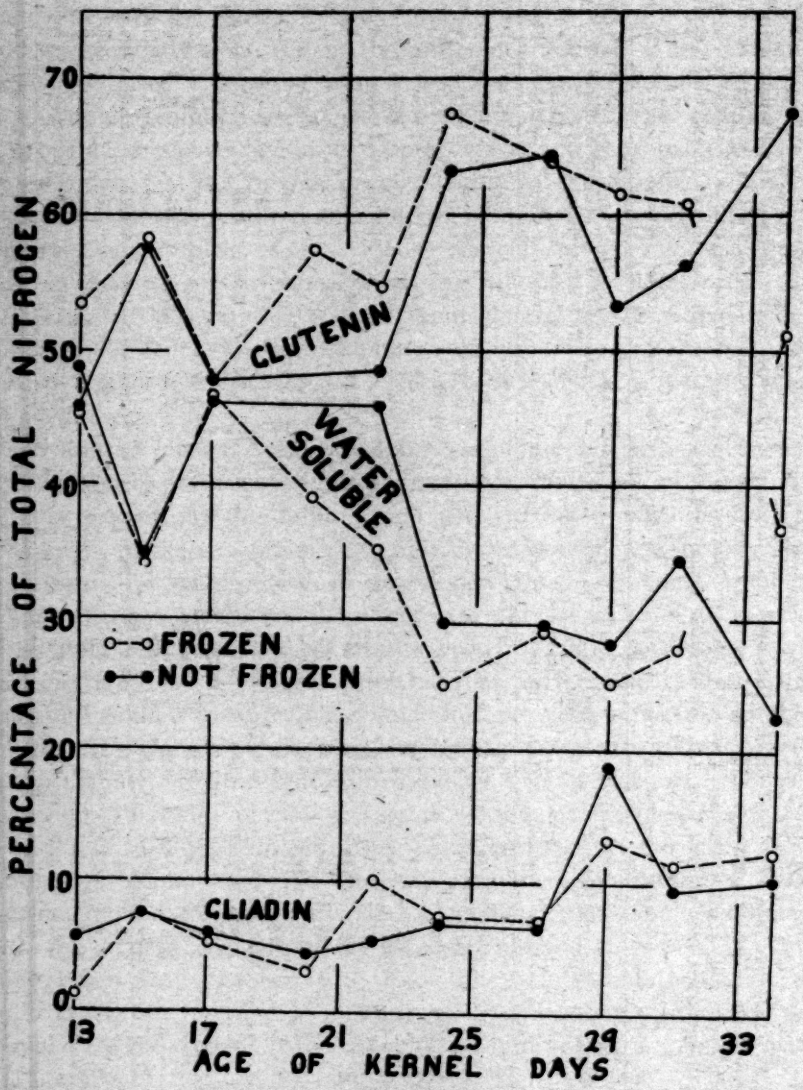

Fig. 4. Composition of the auto-digestion mixtures at the end of 10 weeks. Compare with Figure 5, where water extraction was substituted for 5 per cent potassium sulphate extraction. Compare also with Figure 2, which gives the composition at the start.

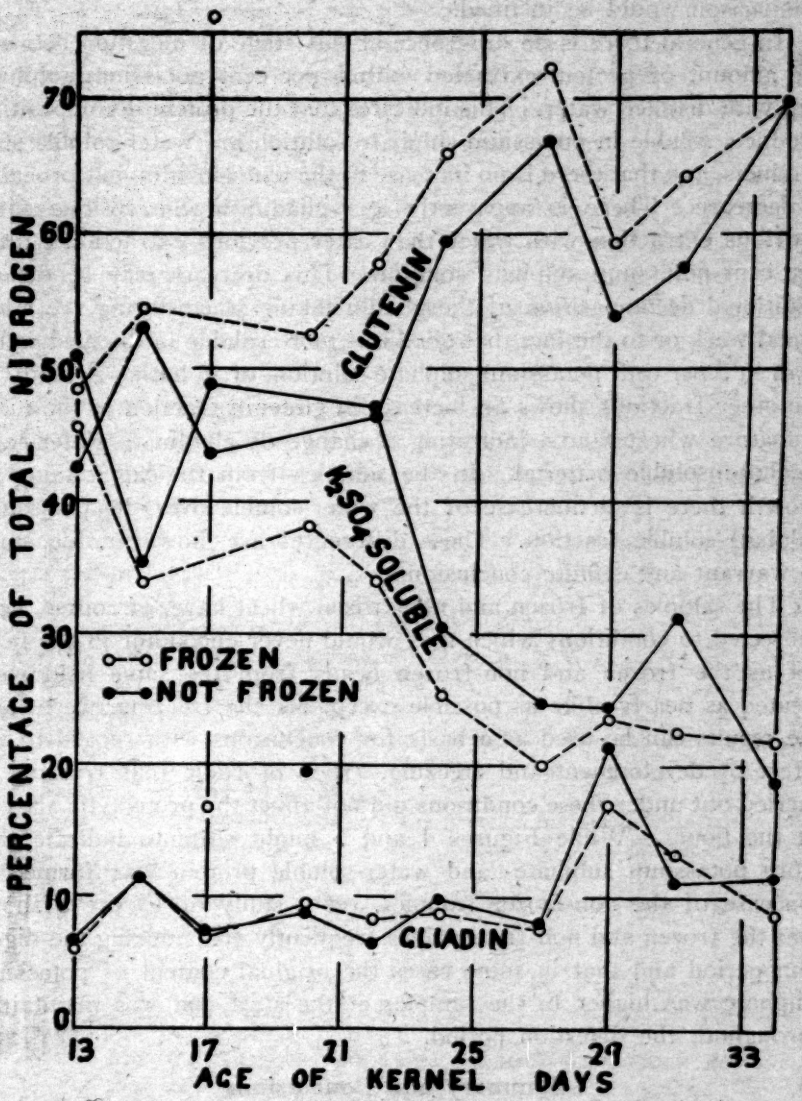

Fig. 5. Composition of the auto-digestion mixtures at the end of 11 weeks. Compare with Figure 4, where 5 per cent potassium sulphate extraction is used in place of water extraction.

sulphate series curves were approaching the horizontal and the change per week was not very great in most cases, it is probable that a direct comparison would be justified.

In general there is no difference at this stage of digestion between the amount of protein extracted with 5 per cent potassium sulphate and with distilled water. This indicates that the protein decomposition products soluble in potassium sulphate solution are water-soluble split products, and that there is no increase in the true globulin, but probably a decrease. There is apparently less gliadin in the residue after previous extraction with water than after previous extraction with 5 per cent potassium sulphate solution. This decrease may be due to additional decomposition of the gliadin taking place during the additional week or to the fact that gliadin is more soluble in distilled water than in 5 per cent potassium sulphate solution, or to both. A study of the other fractions shows an increase in glutenin fraction in the more immature wheat flours, indicating a change of gliadin to water- and alcohol-insoluble material. In the samples from the later stages of growth there is an increase of the water-soluble over the potassium sulphate-soluble fraction. These differences are, however, too small to warrant any definite conclusions.

The samples of frozen and non-frozen wheat have, of course, been subjected to conditions which they would never encounter in the field, yet as the frozen and non-frozen heads from the same field were treated as nearly alike as possible except for the freezing, we believe the results can be used as a basis for conclusions with regard to the effect of development and freezing. It is probable that freezing as carried out under these conditions did not affect the proteolytic activity of the flours. While Figures 4 and 5 might seem to indicate that more potassium sulphate- and water-soluble protein was formed in the case of the non-frozen samples, yet a study of Figure 3 shows that the frozen and non-frozen lines frequently cross during the digestion period and that in some cases the original content of potassium sulphate was higher in the samples at the start and was maintained throughout the digestion period.

Summary and Conclusions

1. Protein fraction analysis of flour milled from wheat harvested at various stages of growth, as previously described, indicates no change in the glutenin, an increase in the gliadin, and a decrease in the 5 per cent potassium sulphate soluble and amino nitrogen as the kernel develops.

2. Subjection of the immature wheat to freezing temperatures by the method described had no effect on the protein fractions as compared with the non-frozen wheat.

3. The total protein of the flour milled from wheat harvested at various stages of maturity did not differ greatly altho a slight decrease followed by an increase is indicated over the range studied.

4. The proteolytic enzymes of wheat flour are capable of digesting the flour protein if given sufficient time in which to act.

5. Auto-digestion of flour for a considerable period of time apparently produces no decrease in the glutenin, causes a decrease in the gliadin, and an increase in the 5 per cent potassium sulphate-soluble and amino nitrogen fractions. The decrease in the gliadin fraction is not all accounted for by the increase in the potassium sulphate-soluble fraction but also shows up as an increase in the glutenin fraction.

6. The decrease in the gliadin fraction is apparently independent of the stage of kernel development, but the percentage increase in the potassium sulphate-soluble fraction is greater in the more immature stages of growth.

7. Freezing as carried out under these conditions apparently affects the proteolytic activity of the flours very little if at all.

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